ISSN 1346-7565

Acta Phytotax. Geobot. 67 (3): 133–146 (2016)
doi: 10.18942/apg.201609

Hybrid Origin of the Apogamous Fern *Dryopteris hondoensis* (Dryopteridaceae)

Kiyotaka Hori[1,*], Yasuyuki Watano[2] and Noriaki Murakami[1]

[1] Makino Herbarium, Tokyo Metropolitan University,1-1 Minamiosawa, Hachioji, Tokyo 192-0397, Japan. [*]hori-kiyotaka@ed.tmu.ac.jp (author for correspondence); [2]Department of Biology, Graduate School of Science, Chiba University, 1-33 Yayoi-cho, Inage, Chiba 263-8522, Japan

We report that the triploid apogamous species *Dryopteris hondoensis* Koidz. shares its sequences with the *D. erythrosora* complex (*D. erythrosora, D. caudipinna* and *D. koidzumiana*) and *D. chinensis* at two nuclear markers (*PgiC* and *GapCp*). This is the first report of reticulate evolution between subg. *Erythrovariae* sect. *Erythrovariae*, to which *D. erythrosora*, *D. caudipinna* and *D. koidzumiana* and *D. hondoensis* belong, and sect. *Aemulae* of subg. *Dryopteris*, to which *D. chinensis* belongs. Apogamous species of *Dryopteris* may hybridize with distantly related species congeners.

Key words: Dryopteridaceae, *Dryopteris chinensis*, *Dryopteris caudipinna*, *GapCp*, *PgiC*, *rbcL*, reticulate evolution

Apogamy, or agamospory in ferns, is a type of asexual reproduction in which unreduced spores are formed. The resultant gametophytes produce sporophytes of the next generation without fertilization (Manton 1950). Apogamous reproduction is common in ferns. Approximately 10% of all species of ferns (Lovis 1977) and 13% of the taxa of Japanese pteridophytes with known mode of reproduction are reported to exhibit apogamous reproduction (Takamiya 1996). In apogamous species, all offspring from a parent are expected to be clonal with a small amount of genetic variation. Interestingly, Darnaedi *et al*. (1990) reported that *Dryopteris yakusilvicola* Sa. Kurata, a triploid apogamous species of recent hybrid origin, showed no allozyme variation within the 56 individuals they examined. They considered *D. yakusilvicola* to have originated from a single hybrid event between the tetraploid sexual species *D. sparsa* and the diploid sexual species *D. sabaei*.

This phenomenon, however, is not common. Despite the clonal nature of apogamous reproduction, many apogamous species of ferns exhibit extensive morphological and genetic variation and often form species complexes in which morphological species are difficult to distinguish because of their continuous variation (Watano & Iwatsuki 1988, Suzuki & Iwatsuki 1990, Lin *et al*. 1995, Grusz *et al*. 2009, Hori *et al*. 2014). This is because apogamous species of ferns can hybridize with other sexual species to produce apogamously reproducible offspring (Lin *et al*. 1995, Ebihara *et al*. 2012, Lee & Park 2013, Hori *et al*. 2014).

Lee and Park (2013) recently attempted to assess reticulate evolution in subg. *Erythrovariae* sect. *Variae* sensu Fraser-Jenkins (1986; the *Dryopteris varia* complex) in Korea. They reported that *D. sacrosancta* (subg. *Erythrovariae*, sect. *Variae*) and *D. chinensis* (subg. *Dryopteris*, sect. *Aemulae* Fraser-Jenk.) shared the same chloroplast DNA (cpDNA) haplotype. Subsequently, Hori *et al*. (2014) reported triploid apogamous individuals of the *D. varia* complex to have three *PgiC* sequences, which can be considered homeologs, on homeologous chromosomes, but not on homologous chromosomes. They reported that *D.*

chinensis and *Dryopteris sacrosancta* and *D. kobayashii*, which Serizawa (2009) separated from *D. sacrosancta s.l.*, shared the same nuclear *PgiC* sequences. The data suggest that apogamous *D. sacrosancta s.s.* and *D. kobayashii* are hybrids between the *D. varia* complex and *D. chinensis*, which are distantly related based on the cpDNA tree (Ebihara 2011, Hori *et al*. 2014).

Furthermore, Hori *et al*. (2014) reported that *Dryopteris pacifica* (subg. *Erythrovariae*, sect. *Variae*) and the *D. erythrosora* complex (subg. *Erythrovariae*, sect. *Erythrovariae*) also shared the same *PgiC* sequences, suggesting that hybridization with members of the *D. erythrosora* complex contributed to the diversification of the apogamous species of the *D. varia* complex. Thus, these results indicate that phylogenetically wider taxon sampling is necessary to understand reticulate evolution in apogamous ferns.

Dryopteris hondoensis Koidz is a triploid apogamous species of sect. *Erythrovariae*, distributed in China, Korea and Japan (Honshu, Shikoku, Kyushu). It is distinguished from other members section *Erythrovariae* by its yellowish green, dull lamina and mostly plane scales on the rachis of the pinnae (Iwatsuki 1995). In this study, we determined *D. hondoensis* to be composed of nuclear DNA sequences from the *D. erythrosora* complex (*D. erythrosora*, *D. caudipinna* and *D. koidzumiana*) of sect. *Erythrovariae* and those from *D. chinensis* of sect. *Aemulae*. This is the first report of reticulate evolution between subg. *Erythrovariae* sect. *Erythrovariae* and subg. *Dryopteris* sect. *Aemulae*.

Materials and Methods

Plant Materials

Leaf samples were collected from six individuals of *Dryopteris hondoensis* and from possibly related sexual species in *Dryopteris* sections *Aemulae*, *Erythrovariae* and *Variae*, mainly from our field surveys in nine prefectures in Japan (Appendix). Their ploidy levels and reproductive modes are shown in Table 1. The reproductive mode of several individuals in the samples was estimated by counting the number of spores per sporangium (32, apogamous; 64, sexual) according to Manton (1950). All voucher specimens are deposited in MAK and/or TNS.

Ploidy analyses

Ploidy analyses followed the methods of Hori *et al*. (2014). To determine ploidy level, the DNA content (2C value) of each nucleus extracted from fresh pinnae was measured once per sample by flow cytometry using a Cyflow Ploidy Analyzer PA-II (Partec, Munster, Germany) and a Cystain UV Precise P kit (Partec). Approximately 100 mm^2 of each pinna was torn into several pieces and finely chopped with a razor blade in 0.25 mL of nucleus extraction buffer from the kit. Then, 0.8 mL of staining solution from the kit was added to the chopped tissues. The crushed tissue and buffers were filtered through a 30-μm nylon mesh (Partec) before analysis. Approximately 25 mm^2 of fresh leaf tissues of *Nicotiana tabacum* L. (2C value = 11.71 pg., Narayan 1987) was used as an internal standard.

Molecular analyses

For molecular analyses, a small amount of leaf tissue was dried in plastic bags, 20 cm × 10 cm, using silica gel. Subsequently, total DNA was extracted from the dried leaves using cetyltrimethylammonium bromide solution according to the method of Doyle & Doyle (1987).

The plastid gene *rbcL* was used in this study as the cpDNA marker. Polymerase chain reaction (PCR) amplification of *rbcL* was performed using the primers aF and cR of Hasebe *et al*. (1994). PCR entailed an initial denaturation step at 95°C for 10 min; followed by 35 cycles of denaturation, annealing, and elongation steps at 98°C for 10 s, 50°C for 10 s, and 72°C for 7 s, respectively; and a final extension step at 72°C for 7 min.

The nuclear gene *PgiC*, including exons 14–16 and introns 14–15, and *GapCp* (short) exons 9–10 were chosen as the nuclear DNA (nrDNA) markers. The *PgiC* fragment was amplified using the primers 14F and 16R of Ishikawa *et al*. (2002). The *GapCp* fragment was amplified using the

TABLE 1. The ploidy levels and reproductive modes of the materials species in this study.

Species	Ploidy	Reproductive mode	References
Dryopteris hondoensis	triploid	apogamous	Hirabayashi (1966, 1967), Mitui (1967, 1968)
D. erythrosora	triploid	apogamous	Hirabayashi (1966, 1967, 1974), Kurita (1961), Mitui (1968)
D. caudipinna	diploid	sexual	Hirabayashi (1970)
D. koidzumiana	diploid	sexual	Hirabayashi (1969), Mitui (1967, 1968)
D. kinkiensis	tetraploid	sexual	Hirabayashi (1967)
D. chinensis	diploid	sexual	Hori *et al*. (2015a)
	triploid	apogamous	Hirabayashi (1966, 1974), Kurita (1961), Mitui (1968)
	tetraploid	apogamous	Weng (1989), Nakato *et al*. (1995)
D. protobissetiana	diploid	sexual	Hori *et al*. (2015b)
D. saxifraga	diploid	sexual	Hirabayashi (1967), Lin *et al*. (1995), Mitui (1975)
	triploid	apogamous	Lee *et al*. (2006)
D. sordidipes	diploid	sexual	Hirabayashi (1969, 1974), Mitui (1967, 1968)
D. varia	diploid	sexual	Ebihara *et al*. (2014)
	diploid	apogamous	Hirabayashi (1966, 1967, 1974), Lin *et al*. (1995)
	triploid	apogamous	Hirabayashi (1970), Mitui (1966, 1968)
	tetraploid	sexual	Tsai & Shieh (1975, 1985)
D. gymnophylla	diploid	sexual	Kurita (1966), Hirabayashi (1969)
D. sabaei	diploid	sexual	Darnaedi & Iwatsuki (1987), Hirabayashi (1966, 1974), Kurita (1962), Mitui (1968)

primers 132F (5′-GTGCTTCCGGAGTTAAATGG-3′) and 488R (5′-CAACATCATCTTCGGTGTATCC-3′), which were newly developed in this study. PCR entailed an initial denaturation step at 95°C for 3 min; followed by 40 cycles of denaturation, annealing, and elongation at 98°C for 10 s, 58°C for 5 s, and 72°C for 5 s, respectively; and a final extension step at 72°C for 1 min. PCR amplification was performed using PrimeSTAR Max DNA Polymerase (Takara, Kyoto, Japan) on a Model 9700 thermal cycler (Applied Biosystems, Foster City, CA, USA).

The nucleotide sequences of *rbcL* were determined by direct sequencing. For sequencing *rbcL*, aF, aR, cR (Hasebe *et al*. 1994), and D. paci-bf (Hori *et al*. 2014) primers were used.

PCR-single-strand conformation polymorphism (SSCP) analysis was performed to examine allelic variation at the nuclear marker level for each individual, following the method of Hori *et al*. (2014). Electrophoresis was performed using MDE gel solution (Lonza) with 2% glycerol at 18°C for 16 h at 350 V for *PgiC* and 2% glycerol at 15°C for 9.5 h at 300 V for *GapCp*.

To sequence the bands separated on the SSCP gels, pieces of the DNA bands in the dried gel were peeled off and incubated in 50 μL of TE buffer (10 mM Tris-HCl and 1 mM EDTA, pH 8.0) at 37°C overnight. The supernatant solution was used as a template for further PCR amplification. Before sequencing, the PCR products were purified using Illustra ExoStar 1-Step (GE Healthcare, WI, USA) and used as templates for direct sequencing. The reaction mixtures for sequencing were prepared using a BigDye Terminator v.3.1 Cycle Sequencing Kit (Applied Biosystems). The reaction mixtures were analyzed using an ABI 3130 Genetic Analyzer (Applied Biosystems).

All plant samples were classified based on their PCR-SSCP banding patterns. The genomic constitution of each band pattern was identified by determining the nucleotide sequences of each DNA band separated on the SSCP gel.

Phylogenetic analyses

For phylogenetic analyses, only one sequence representing each haplotype for cpDNA (*rbcL*)

and each allele for nrDNA (*GapCp*, *PgiC*) was used in our datasets. The cpDNA and nrDNA sequences were aligned using MUSCLE Citation (Edgar 2004) and analyzed separately by neighbor-joining (NJ), maximum parsimony (MP), or maximum likelihood (ML) analyses using MEGA version 6 (Tamura *et al*. 2013). The NJ tree was obtained using the p-distance method (Nei & Kumar 2000). The data are expressed as the number of base differences per site. All ambiguous positions were removed from each sequence pair. The MP tree was obtained using the subtree-pruning-regrafting algorithm (Nei & Kumar 2000) at search level 1, in which the initial trees were obtained by the random addition of sequences (10 replicates). In the ML analysis, the best-fitting model of sequence evolution for each DNA region was selected using MEGA version 6 (Tamura *et al*. 2013). The *GapCp* tree was constructed using the T92+G model, the *PgiC* tree was constructed using the HKY model, and the *rbcL* tree was constructed using the K2+G model. The initial trees for the heuristic search were obtained automatically by applying neighbor-join and BioNJ algorithms to a matrix of pairwise distances estimated using the maximum composite likelihood approach and then selecting the topology with superior log likelihood value. The indels were treated as missing characters in the MP and ML analyses. The bootstrap method with 1000 replications was employed to estimate the confidence levels of monophyletic groups. *Dryopteris sabaei* (sect. *Nephrocystis*, Fraser-Jenk.), *D. fragrans* (quoted from DDBJ) and two species of *Polystichum* (quoted from DDBJ) were used as outgroups.

Morphological observation

The morphology of *Dryopteris hondoensis*, *D. chinensis*, *D. erythrosora*, *D. caudipinna* and *D. koidzumiana* was compared, particularly focusing on differences in the color and sheen of the lamina, shape of the lowest basiscopic pinnule, and shape of the scales.

Results

Ploidy analyses

The genome size of each species in this study was estimated as follows: *Dryopteris koidzumiana* (diploid sexual species; Hirabayashi 1969, Mitui 1967, 1968), 17.14 pg ($N = 1$); *D. caudipinna* (diploid sexual species; Hirabayashi 1970), 18.04 ± 0.19 pg ($N = 12$); *D. erythrosora* (triploid apogamous species; Hirabayashi 1966, 1967, 1974, Kurita 1961, Mitui 1968), 23.48 ± 0.94 pg ($N = 5$); *D. hondoensis* (triploid apogamous species; Hirabayashi 1966, 1967, Mitui 1967, 1968), 21.44 ± 0.53 pg ($N = 4$); and *D. kinkiensis* (tetraploid sexual species; Hirabayashi 1967), 28.07 ± 0.92 pg ($N = 3$). The genome sizes of *D. erythrosora* and *D. hondoensis* (only the triploid apogamous cytotype has been reported for this species in Japan, Table 1) were less than two-thirds of those of *D. caudipinna* and *D. koidzumiana* (diploid sexual species), but they were larger and smaller than those of *D. kinkiensis* (tetraploid sexual species). Those samples were therefore estimated to be triploid apogamous.

Sequence variation of rbcL *and molecular phylogenetic trees based on variation*

In *Dryopteris hondoensis* and possibly related species, 12 types of *rbcL* sequences were found (Fig. 1, Appendix). The GenBank accession numbers of the obtained sequences are shown in Appendix. Among the 1205 sites, 105 (9%) were variable and 42 (3%) were parsimoniously informative. NJ, MP, and ML analyses uncovered phylogenetic trees with similar topology. The ML tree (highest log likelihood = −2451.5660) with bootstrap percentages (BPs) of NJ/MP/ML analyses are shown in Fig. 1. *Dryopteris hondoensis*, *D. erythrosora*, *D. caudipinna*, and *D. koidzumiana* displayed *rbcL* sequences belonging to the same clade.

Sequence variation of PgiC *and phylogenetic trees base on variation*

In each of the samples, several different sequences of nuclear *PgiC* were detected by SSCP

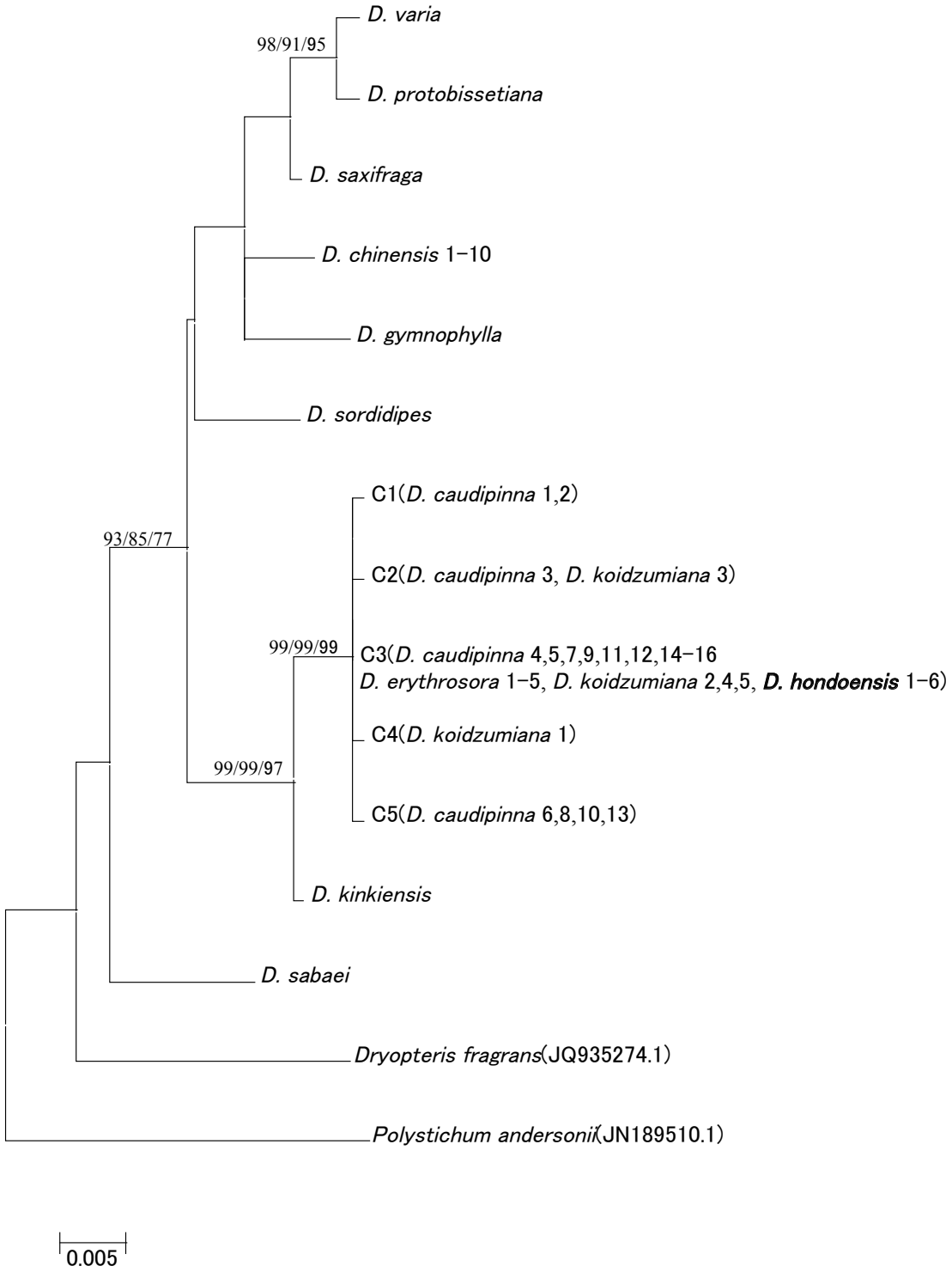

FIG. 1. ML tree (highest log likelihood = −2451.5660) based on sequence variation of plastid gene *rbcL* with BPs (>70) of NJ/MP/ML analyses.

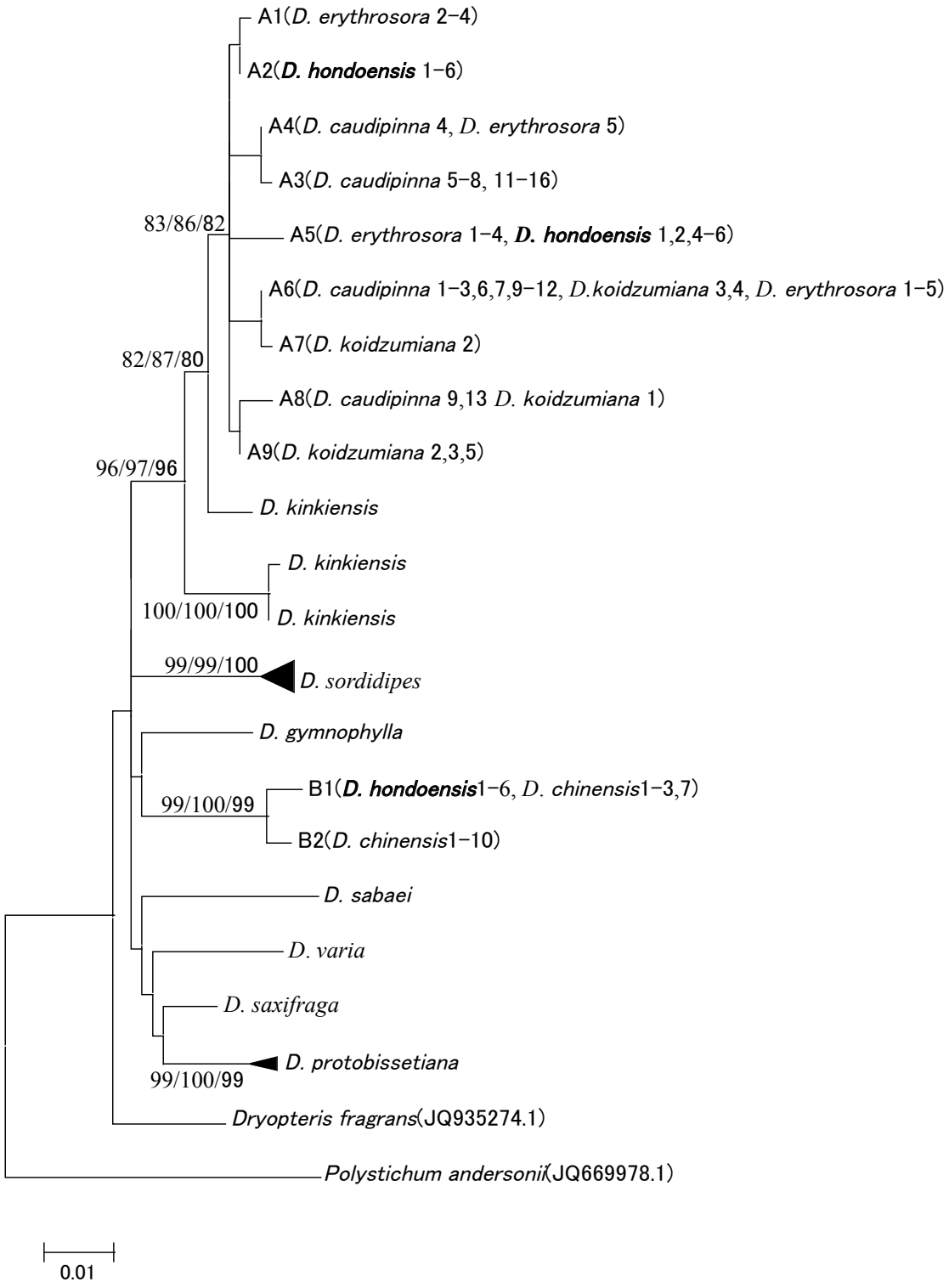

FIG. 2. ML tree (highest log likelihood = −2061.8095) based on sequence variation of nuclear gene *PgiC* with BPs (>70) of NJ/MP/ML analyses is shown.

analyses. The number of different sequences was always the presumed ploidy level of the sample or less. Twenty seven different sequences of *PgiC* were identified in the samples of *Dryopteris hondoensis* and in other species of *Dryopteris* analyzed in the present study. The length of the sequences varied from 607 to 661 bp. The data matrix for phylogenetic analyses included 672 characters after editing, of which 153 (23%) were variable and 68 (10%) were parsimoniously informative. The ML tree (highest log likelihood = −2061.8095) with BPs of NJ/MP/ML analyses is shown in Fig. 2. Two monophyletic groups (A and B) were recognized, each of which was supported by a high BP. Group A contained the *PgiC* sequences of *D. erythrosora*, *D. caudipinna*, and *D. koidzumiana*, whereas Group B contained sequences of *D. chinensis. Dryopteris hondoensis* contained both group A and group B sequences. Some samples of *D. hondoensis* had two sequences of group A and one sequence of group B; others had one sequence of group A and one sequence of group B (Table 2).

Sequence variation of GapCp *and the phylogenetic tree based on the variation*

In most of the samples, several different sequences of nuclear *GapCp* were detected by SSCP analyses. In total, 21 different sequences were identified in the samples of *Dryopteris hondoensis* and some *Dryopteris* species analyzed in the present study. The length of the sequences varied from 301 to 350 bp. The data matrix for phylogenetic analyses included 360 characters after editing, of which 87 (24%) were variable and 48 (13%) were parsimoniously informative. The ML tree (highest log likelihood = −1166.5740) with BPs of NJ/MP/ML analyses is shown in Fig. 3. Four monophyletic groups (A, B, C, and D) were recognized, each of which was supported by a high BP. Group A contained the *GapCp* sequences of *D. chinensis*, and Groups B–D contained those of *D. erythrosora*, *D. caudipinna*, and *D. koidzumiana*. *Dryopteris hondoensis* had three sequences, one each from groups A, B, and D (Table 2). D_2 and D_3 were distinguished by the positions of indels, but they are not distinguished on the molecular tree because indels were excluded in the phylogenetic analyses.

The estimated genotype of each sample is summarized in Appendix. It must be noted that our current method cannot distinguish differences in gene dosage in polyploids; therefore, $A_1A_1C_1$, A_1C_1, and $A_1C_1C_1$ were not distinguishable and were thus indicated as A_1C_1*.

Morphological observation

The morphology of *Dryopteris hondoensis*, *D. chinensis*, and species in the *D. erythrosora* complex (*D. erythrosora*, *D. caudipinna* and *D. koidzumiana*) is shown in Figs. 4–6 and Table 3. The adaxial lamina surface of *D. hondoensis* is yellowish green and dull, fresh green and dull in *D. chinensis*, and dark-green and shiny in the *D. erythrosora* complex (Fig. 4). The most basal basiscopic pinnule of the lowest pinnae of *D. hondoensis* is slightly more shortened than the second-most basiscopic pinnule. Those of the *D. erythrosora* complex is clearly shortened than the second most basiscopic pinnule. Those of *D. chinensis* is more elongated than the second most basiscopic pinnule. (Fig. 5). Iwatsuki (1995) described the scales of *D. hondoensis* as being mostly plane, with bullate scales intermixed on the rachis of the pinna rachis. However, this is incorrect. These two types of scales are not intermixed on the pinna rachis. The scales of *D. hondoensis* are bullate distally on the rachis of the pinnae (Fig. 6c), but plane on the basal portion of the pinnae (Fig. 6g).

Discussion

Dryopteris hondoensis has never been considered to be closely related to *D. chinensis*. Fraser-Jenkins (1986) included *D. chinensis* in *Dryopteris* subgen. *Dryopteris* sect. *Aemulae,* whereas he included *D. caudipinna*, *D. erythrosora*, *D. koidzumiana*, and *D. hondoensis* in *Dryopteris* subgen. *Erythrovariae* sect. *Erythrovariae*. Thus, he (Fraser-Jenkins, 1986) did not discuss the similarity in morphological characteristics between these species.

Our *PgiC* and *GapCp* analyses, however, sug-

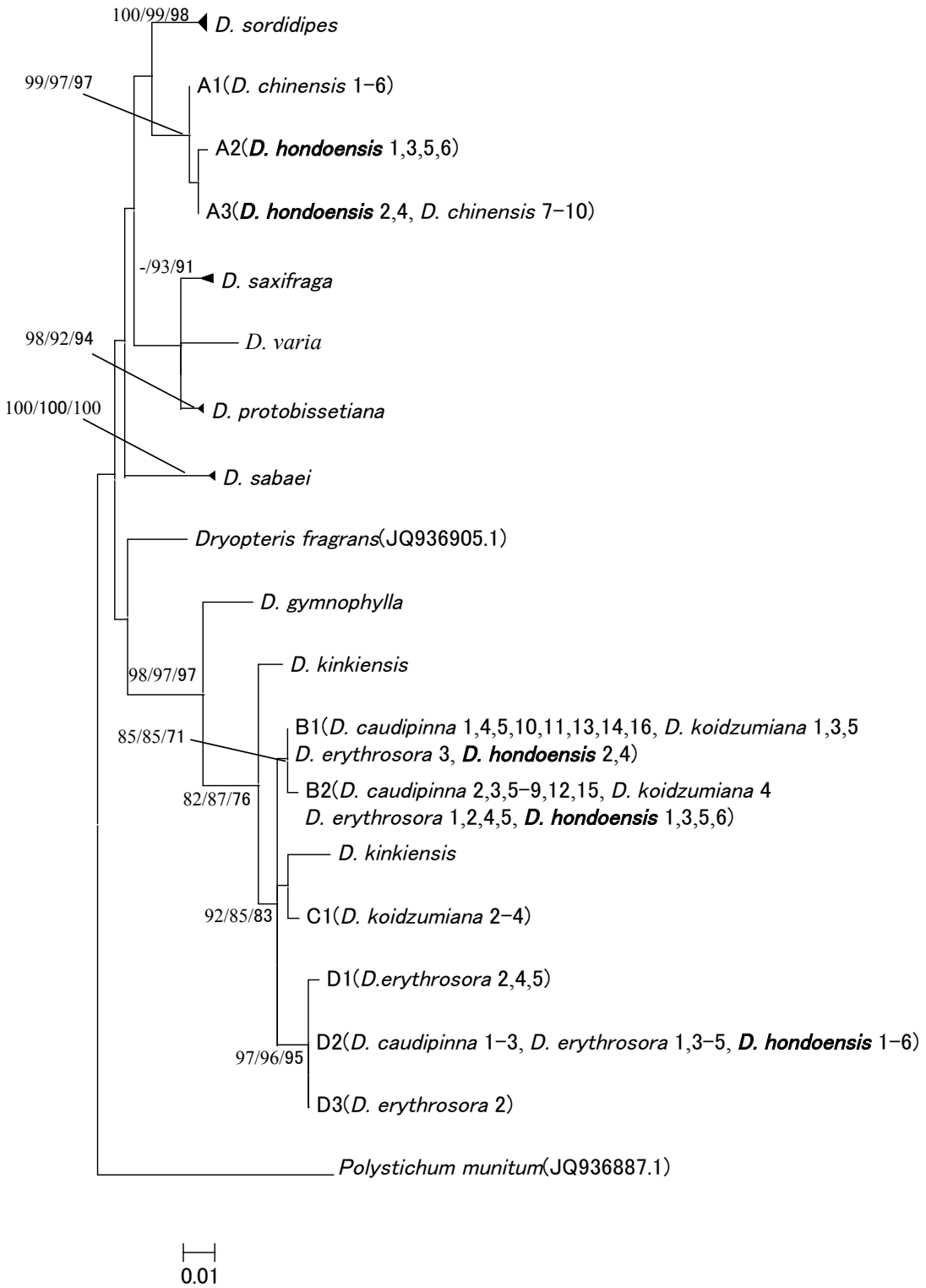

FIG. 3. ML tree (highest log likelihood = −821.3017) based on sequence variation of nuclear gene *GapCp* with BPs (>70) of NJ/MP/ML analyses is shown.

TABLE 2. Ploidy level, reproductive mode and constitution of nuclear maekers (*PgiC, GapCp*).

Species	*PgiC* type	*GapCp* type	Ploidy level, reproductive mode	number of samples	locality
D. caudipinna 1	A_6A_6	B_1D_2	2x / sexual	1	Hyogo pref.
D. caudipinna 2,3	A_6A_6	B_2D_2	2x / sexual	2	Hyogo pref.
D. caudipinna 4	A_4A_4	B_1B_1	2x / sexual	1	Kanagawa pref.
D. caudipinna 5	A_3A_3	B_1B_2	2x / sexual	3	Kanagawa pref.
D. caudipinna 6,7,12	A_3A_6	B_2B_2	2x / sexual	3	Kanagawa pref.
D. caudipinna 9	A_6A_8	B_2D_2	2x / sexual	1	Kanagawa pref.
D. caudipinna 10	A_6A_6	B_1B_1	2x / sexual	1	Kanagawa pref.
D. caudipinna 11	A_3A_6	B_1B_1	2x / sexual	1	Kanagawa pref.
D. caudipinna 13	A_3A_8	B_1B_1	2x / sexual	1	Kanagawa pref.
D. caudipinna 14,16	A_3A_3	B_1B_1	2x / sexual	2	Kanagawa pref.
D. koidzumiana 1	A_8A_8	B_1B_1	sexual	1	Kagoshima pref., Yakushima
D. koidzumiana 2	A_7A_9	C_1C_1	2x / sexual	1	Kagoshima pref., Yakushima
D. koidzumiana 3	A_6A_9	B_1C_1	sexual	1	Kagoshima pref., Yakushima
D. koidzumiana 4	A_6A_6	B_2C_1	sexual	1	Kagoshima pref., Yakushima
D. koidzumiana 5	A_9A_9	B_1B_1	sexual	1	Kagoshima pref., Yakushima
D. erythrosora 1	A_5/A_6	B_2/D_2	3x	1	Miyazaki pref.
D. erythrosora 2	$A_1A_5A_6$	$B_2D_1D_3$	3x	1	Wakayama pref.
D. erythrosora 3	$A_1A_5A_6$	B_1/D_2	3x	1	Wakayama pref.
D. erythrosora 4	$A_1A_5A_6$	$B_2D_1D_2$	3x	1	Wakayama pref.
D. erythrosora 5	$A_2A_4A_6$	$B_2D_1D_2$	3x / apogamous	1	Tokyo-to.
D. hondoensis 1,5,6	$A_2A_5B_1$	$A_2B_2D_2$	3x / apogamous	3	Tokyo-to., Wakayama pref.
D. hondoensis 2,4	$A_2A_5B_1$	$A_3B_1D_2$	3x	2	Tokyo-to.
D. hondoensis 3	A_2/B_1	$A_2B_2D_2$	3x	1	Miyazaki pref.
D. chinensis 7	B_1B_2	A_3A_3	2x / sexual (Hori *et al*. 2015)	1	Miyazaki pref.
D. chinensis 4-6	B_2B_2	A_1A_1	2x / sexual (Hori *et al*. 2015)	3	Miyazaki pref.
D. chinensis 8-10	B_2B_2	A_3A_3	2x / sexual (Hori *et al*. 2015)	3	Miyazaki pref.
D. chinensis 1-3	B_1/B_2	A_1	apogamous	2	Tokyo-to., Wakayama pref.

TABLE 3. Morphological differences between *Dryopteris hondoensis* and its related species.

	D. hondoensis	*D. chinensis*	*D. caudipinna*	*D. koidzumiana*	*D. erythrosora*
Color of lamina	**yellowish green, dull**	fresh green, dull	dark green, shiny	dark green, shiny	dark green, shiny
The most basal basiscopic pinnule of the lowest pinna	**elongate a little than the second one**	elongate than the second one	shorten than the second one	shorten than the second one	shorten than the second one
Scales on distal positions of pinna rachis	**bullate**	flat	bullate	bullate	bullate
Scales on basal positions of pinna rachis	**flat**	flat	bullate	bullate	bullate

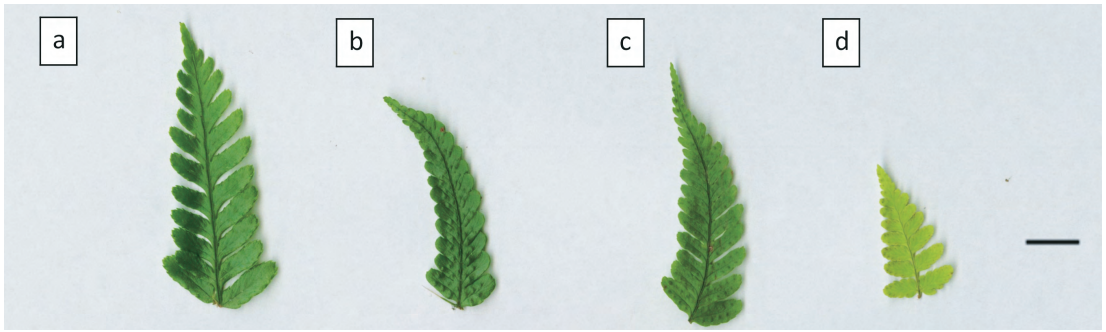

FIG. 4. Color of pinnule of the second most basal pinna. (a: *D. caudipinna*, b: *D. erythrosora*, c: *D. hondoensis*, d: *D. chinensis*; scale = 1 cm)

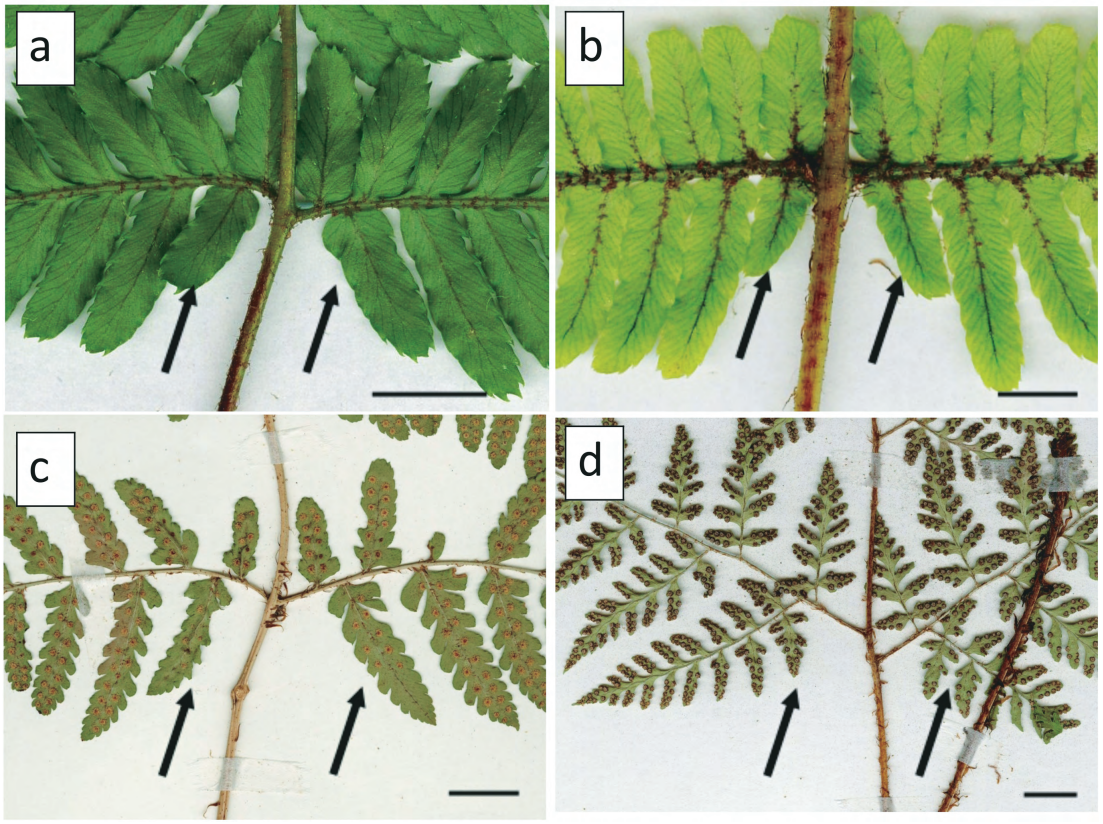

FIG. 5. Most basal basiscopic pinnule of lowest pinnae. (1: *D. caudipinna*, 2: *D. erythrosora*, 3: *D. hondoensis*, 4: *D. chinensis*; scale = 1 cm).

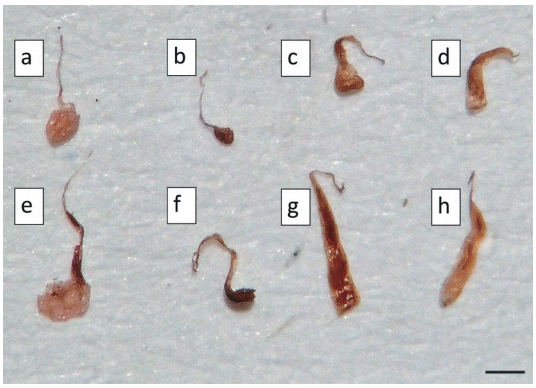

FIG. 6. Scales on distal (a–d) and basal (e–h) positions of rachis of pinna. (a/e: *D. caudipinna*, b/f: *D. erythrosora*, c/g: *D. hondoensis*, d/h: *D. chinensis*; scale = 1 mm)

gested that *Dryopteris hondoensis* is of hybrid origin between *Dryopteris. caudipinna*, *D. koidzumiana*, *D. erythrosora* and *D. chinensis* because it contains DNA sequences matching the clades of those species in the molecular phylogenetic trees (Figs. 2 & 3). Furthermore, *D. hondoensis* possibly originated at least three times because *D. hondoensis* comprises three clones.

The results of the maternally inherited *rbcL* suggests that the maternal parent of *D. hondoensis* was *D. erythrosora* (triploid apogamous), *D. caudipinna* and *D. koidzumiana* (diploid sexual species). Hori *et al*. (2014) suggested that diploid apogamous offspring originating from triploid apogamous individuals through unequal meiosis can be both maternal and paternal species in the reticulate evolution of the *D. varia* complex. Therefore, we have two hypotheses about the origin of *D. hondoensis*. First, triploid apogamous *D. chinensis* (paternal) may result in unequal meiosis and then cross with diploid sexual *D. caudipinna* or *D. koidzumiana* (maternal). In this case, there may have been backcrossing to *D. caudipinna*, *D. koidzumiana* and *D. erythrosora*, because *D. hondoensis* shows two sequences belonging to the *D. erythrosora* complex clades in the trees. Second, triploid apogamous *D. erythrosora* (maternal) may have unequal meiosis and then cross with diploid sexual *D. chinensis* (paternal). Additional nuclear markers are needed to determine the more plausible hypothesis.

Dryopteris hondoensis and *D. chinensis* display clear ecological and morphological differentiation; specifically, *D. hondoensis* is evergreen and has ovate to broadly ovate–subdeltoid lamina, whereas *D. chinensis* is summer green and has pentagonal lamina. They have similar morphological characteristics, however, that coincide with the results from our DNA analyses that suggest a hybrid origin for *D. hondoensis*. *Dryopteris chinensis* has fresh green lamina and lanceolate scales on the stipes while *D. hondoensis* has yellowish green lamina and lanceolate scales on the stipes and basal portion of the rachis of the pinnae. Iwatsuki (1995) did not postulate a hybrid origin for *D. hondoensis* between the *D. erythrosora* complex and *D. chinensis*, even though he listed several natural hybrids as being in Japan.

In our study, we suggest the importance of phylogenetically wider sampling, including distantly related species belonging to other sections, to clarify the origin of apogamous species of ferns and to resolve the reticulate relationships in *Dryopteris*.

We are grateful to the following persons for their assistance in collecting plant materials: Dr. J. Yamashita (Okayama University); S. Kariyama (Kurashiki Museum of Natural History); and Mr. K. Akagi, Y. Inoue, T. Minamitani, K. Mizote, and K. Ohora (Nippon Fernist Club). This study was partly supported by a Grant-in-Aid for JSPS Fellows No. 26-1720 to K. H. and by a Grant-in-Aid for Scientific Research No. 25291089 to N. M.

References

Darnaedi, D. & K. Iwatsuki. 1987. On the structure and systematic position of the fern *Dryopteris yakusilvicola* Kurata. J. Fac. Sci. Univ. Tokyo III 14: 121–136.

Darnaedi, D., M. Kato & K. Iwatsuki. 1990. Electrophoretic evidence for the origin of *Dryopteris yakusilvicola* (Dryopteridaceae). Bot. Mag. Tokyo 103: 1–10.

Doyle, J. A. & J. L. Doyle. 1990. Isolation of plant DNA from fresh tissue. Focus. 12: 13–15.

Ebihara, A. 2011. *RbcL* phylogeny of Japanese pteridophyte flora and implications on intrafamilial systematics. Bull. Nat. Mus. Ser. Nat. Sci. B 37: 63–74.

Ebihara, A., S. Matsumoto & M. Kato. 2012. Origin of *Dryopteris shibipedis* (Dryopteridaceae), a fern species extinct in the wild. J. Plant. Res. 125: 499–505.

Ebihara, A., N. Nakato, S. Matsumoto, Y. S. Chao & L. Y. Kuo. 2014. Cytotaxonomic studies on thirteen ferns of Taiwan. Bull. Nat. Mus. Ser. Nat. Sci. B 40: 19–28.

Edgar, R. C. 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucl. Acids Res. 2004. 32: 1792–1797.

Fraser-Jenkins, C. R. 1986. A classification of the genus *Dryopteris* (Pteridophyta: Dryopteridaceae). Bull. Brit. Mus. Nat. Hist (Bot). 14: 183–218.

Grusz, A. L., M. D. Windham & K. M. Pryer. 2009. Deciphering the origins of apomictic polyploids in the *Chelianthes yavapensis* complex (Pteridaceae). Am. J. Bot. 96: 1636–1645.

Hasebe, M., T. Omori, M. Nakazawa, T. Sano, M. Kato & K. Iwatsuki. 1994. *RbcL* gene sequences provide evidence for the evolutionary cytotypes of leptosporangiate ferns. Proc. Natl. Acad. Sci. USA. 91: 5730–5734.

Hirabayashi, H. 1966. Chromosome numbers in Japanese species of *Dryopteris* (1). J. Jap. Bot. 41: 11–13.

Hirabayashi, H. 1967. Chromosome numbers in Japanese species of *Dryopteris* (2). J. Jap. Bot. 42: 44–48.

Hirabayashi, H. 1969. Chromosome numbers in Japanese species of *Dryopteris* (3). J. Jap. Bot. 44: 85–95.

Hirabayashi, H. 1970. Chromosome numbers in Japanese species of *Dryopteris* (4). J. Jap. Bot. 45: 11–19.

Hirabayashi, H. 1974. Cytogeographic Studies on *Dryopteris* of Japan. Hara Shobo, Tokyo.

Hori, K., A. Tono, K. Fujimoto, J. Kato, A. Ebihara, Y. Watano & N. Murakami. 2014. Reticulate evolution in the apogamous *Dryopteris varia* complex (Dryopteridaceae, subg. *Erythrovariae*, sect. *Variae*) and its related sexual species in Japan. J. Plant. Res. 127: 661–684.

Hori, K., M. Matsumoto, A. Ebihara, N. Nakato & N. Murakami. 2015a. Geographical distribution of sexual and apogamous types of *Dryopteris chinensis* (Dryopteridaceae) in Japan. Acta Phytotax. Geobot. 66: 35–45.

Hori, K., A. Ebihara, N. Nakato & N. Murakami 2015b. *Dryopteris protobissetiana* (Dryopteridaceae), a new diploid sexual species of the *Dryopteris varia* complex (subg. *Erythrovariae*, sect. *Variae*) from Yakushima, Kagoshima, Japan. Acta Phytotax. Geobot. 66: 47–57.

Ishikawa, H., Y. Watano, K. Kano, M. Ito & S. Kurita. 2002. Development of primer sets for PCR amplification of the PgiC gene in ferns. J. Plant. Res. 115: 65–70.

Iwatsuki, K. 1995. Pteridophyta. *In*: Iwatsuki, K., T. Yamazaki, D. E. Boufford & H. Ohba. (eds.), Flora of Japan, vol. I, pp. 1–239. Kodansha, Tokyo.

Kurita, S. 1961. Chromosome numbers of some Japanese ferns (II). Bot. Mag. Tokyo 74: 395–401.

Kurita, S. 1962. Chromosome numbers of some Japanese ferns (III). J. Coll. Arts Sci. Chiba Univ. 3: 463–468, with 2 plates.

Kurita, S. 1966. Chromosome numbers of some Japanese ferns (6). J. Jap. Bot. 41: 173–180 (in Japanese with English Summary).

Lee, S.-J., Y.-D. Kim, Y. Suh, S.-K. Lee & C.-W. Park. 2006. Morphological and chromosomal variation of the *Dryopteris varia* (L.) Kuntze complex (Dryopteridaceae) in Korea. Plant Syst. Evol. 262: 37–52.

Lee, S.-J. & C.-W. Park. 2013. Relationships and origins of the *Dryopteris varia* (L.) Kuntze species complex (Dryopteridaceae) in Korea inferred from nuclear and chloroplast DNA sequences. Biochem. Syst. Ecol. 50: 371–382.

Lin, S.-J., M. Kato & K. Iwatsuki. 1995. Electrophoretic variation of the apogamous *Dryopteris varia* group (Dryopteridaceae). J. Plant. Res. 108: 451–456.

Lovis, J. D. 1977. Evolutionary patterns and processes in ferns. Adv. Bot. Res. 4: 229–415.

Manton, I. 1950. Problems of cytology and evolution in the Pteridophyta. Cambridge University Press, Cambridge.

Mitui, K. 1966. Chromosome studies on Japanese ferns (2). J. Jap. Bot. 41: 60–64.

Mitui, K. 1967. Chromosome studies on Japanese ferns (3). J. Jap. Bot. 42: 105–110.

Mitui, K. 1968. Chromosomes and speciation in ferns. Sci. Rep. Tokyo Kyoiku Daigaku Sec. B 13: 285–333.

Mitui, K. 1975. Chromosome numbers of Japanese Pteridophytes. Bull. Nippon Dental Coll., General Edu. 4: 221–271.

Nakato, N., M. Kato & B. D. Liu. 1995. A cytotaxonomic study of some ferns from Jiangsu and Zhejiang Provinces, China. J. Jap. Bot. 70: 194–204.

Narayan, R. K. 1987. Nuclear DNA changes, genome differentiation and evolution in *Nicotiana* (Solanaceae). Plant. Syst. Evol. 157: 161–180.

Nei, M., & S. Kumar. 2000. Molecular Evolution and Phylogenetics. Oxford University Press, New York.

Serizawa, S. 2009. *Dryopteris kobayashii* Kitagawa and *D. sacrosancta* Koidz. Shidekobushi. 1: 97–99 (in Japanese).

Suzuki, T. & K. Iwatsuki. 1990. Genetic variation in agamosporous fern *Pteris cretica* L. in Japan. Heredity. 65: 221–227.

Takamiya, M. 1996. Index to chromosomes of Japanese Pteridophyta (1910–1996). Japan Pteridological Society, Tokyo, Japan.

Tamura, K., G. Stecher, D. Peterson, A. Fillipski & S. Kumar. 2013. MEGA6: Molecular evolutionary genetics analysis version 6. Molec. Biol. Evol. 30: 2725–2729.

Tsai, J. L., & W. C. Shieh. 1975. Chromosome numbers of the fern family Aspidiaceae (sensu Copeland) in Taiwan (1). J. Sci. Engin. 12: 321–334.

Tsai, J. L., & W. C. Shieh. 1985. A cytotaxonomic survey of the fern family Aspidiaceae (sensu Copeland) in Taiwan. J. Sci. Engin. 22: 121–144.

Watano, Y. & K. Iwatsuki. 1988. Genetic variation in the 'Japanese apogamous form' of the fern *Asplenium unilaterale* Lam. Bot. Mag. Tokyo. 101: 213–222.

Weng, R. F. 1989. Cytological observation on *Dryopteris* from East China. *In*: Shing, K. H. & K. U. Kramer (eds.), Proceedings of International Symposium on Systematic Pteridology, pp. 141–146. China Science and Technology Press, Beijing.

Received November 11, 2015; accepted March 9, 2016

APPENDIX. Voucher specimens examined in this study. Genotypes (*GapCp*, *PgiC*) identified by sequencing are in bold, otherwise, genotypes were deduced from comparison of band positions in SSCP gels. When ploidy is unknown, the genotype is in brackets. For samples with unknown genome dosage, unidentified genomes are in italic. *Quoted from Hori *et al*. (2014), **Quoted from Hori *et al*. (2015a), ***Quoted from Hori *et al*. (2015b).

Species	Locality	DNA amount (pg.)	Ploidy level	Reproductive mode	*rbcL* type	*PgiC* type	*GapCp* type	Herbarium no.	
								MAK	TNS
*D. caudipinna*1	Japan, Hyogo pref., Himeji-shi			sex	AB908383*	**A_6A_6(LC093995)**	**B_1D_2(LC094033,LC094035)**	411423	
*D. caudipinna*2	Japan, Hyogo pref., Himeji-shi			sex	AB908383*	A_6A_6(LC093995)	**B_2D_2(LC094034,LC094035)**	411424	
*D. caudipinna*3	Japan, Hyogo pref., Himeji-shi			sex	AB908382*	A_6A_6(LC093995)	B_2D_2(LC094034,LC094035)	411425	
*D. caudipinna*4	Japan, Kanagawa pref., Zushi-shi			sex	AB908384*	**A_4A_4(LC093994)**	**B_1B_1(LC094033)**		1190554
*D. caudipinna*5	Japan, Kanagawa pref., Zushi-shi	18.40	2*x*	sex	AB908384*	**A_3A_3(LC093993)**	**B_1B_1(LC094033,LC094034)**	432377	
*D. caudipinna*6	Japan, Kanagawa pref., Zushi-shi	17.95	2*x*	sex	LC094043	A_3A_6(LC093993,LC093995)	B_2B_2(LC094034)	432378	
*D. caudipinna*7	Japan, Kanagawa pref., Zushi-shi	17.96	2*x*	sex	AB908384*	A_3A_6(LC093993,LC093995)	B_2B_2(LC094034)	432379	
*D. caudipinna*8	Japan, Kanagawa pref., Zushi-shi	16.73	2*x*	sex	LC094043	A_3A_3(LC093993)	B_2B_2(LC094034)	432382	
*D. caudipinna*9	Japan, Kanagawa pref., Zushi-shi	18.54	2*x*	sex	AB908384*	**A_6A_6(LC093995,LC093996)**	**B_2B_2(LC094034)**	432385	
*D. caudipinna*10	Japan, Kanagawa pref., Zushi-shi	18.54	2*x*	sex	LC094043	**A_6A_6(LC093995)**	B_1B_1(LC094033)	432386	
*D. caudipinna*11	Japan, Kanagawa pref., Zushi-shi	17.57	2*x*	sex	AB908384*	**A_3A_4(LC093993,LC093995)**	B_1B_1(LC094033)	432387	
*D. caudipinna*12	Japan, Kanagawa pref., Zushi-shi	17.57	2*x*	sex	AB908384*	A_3A_6(LC093993,LC093995)	B_2B_2(LC094034)	432388	
*D. caudipinna*13	Japan, Kanagawa pref., Zushi-shi	17.84	2*x*	sex	LC094043	**A_3A_6(LC03993,LC093996)**	B_1B_1(LC094033)	432390	
*D. caudipinna*14	Japan, Kanagawa pref., Zushi-shi	18.10	2*x*	sex	AB908384*	A_3A_3(LC093993)	B_1B_1(LC094033)	432392	
*D. caudipinna*15	Japan, Kanagawa pref., Zushi-shi	17.84	2*x*	sex	AB908384*	A_3A_3(LC093993)	**B_2B_2(LC094034)**	432397	
*D. caudipinna*16	Japan, Kanagawa pref., Zushi-shi	19.52	2*x*	sex	AB908384*	A_3A_3(LC093993)	B_1B_1(LC094033)	432401	
*D. koidzumiana*1	Japan, Kagoshima pref., Yakushima Is.			sex	AB908381*	**A_6A_6(LC093999)**	B_1B_1(LC094033)	411078	
*D. koidzumiana*2	Japan, Kagoshima pref., Yakushima Is.	17.14	2*x*	sex	AB920329*	**A_2A_4(LC093998,LC094000)**	**C_1C_1(LC094014)**	413260	
*D. koidzumiana*3	Japan, Kagoshima pref., Yakushima Is.			sex	AB908382*	**A_6A_6(LC093997,LC094000)**	**B_1C_1(LC094014,LC094039)**	413262	
*D. koidzumiana*4	Japan, Kagoshima pref., Yakushima Is.			sex	AB920329*	**A_6A_6(LC093997)**	**B_2C_1(LC094014,LC094040)**	413268	
*D. koidzumiana*5	Japan, Kagoshima pref., Yakushima Is.			sex	AB920329*	**A_6A_6(LC094000)**	**B_1B_1(LC094039)**	413349	
*D. erythrosora*1	Japan, Miyazaki pref., Nobeoka-shi	23.42	3*x*		LC094041	**A_2/A_6(LC094004,LC094005)**	**B_2/D_2(LC094011,LC094013)**	410287	
*D. erythrosora*2	Japan, Wakayama pref., Shinguu-shi	26.12	3*x*		LC094041	$A_1A_5A_6$(LC094001,LC094004,LC094005)	**$B_1D_1D_2$(LC094009,LC094010,LC094013)**	410977	
*D. erythrosora*3	Japan, Wakayama pref., Shinguu-shi	22.40	3*x*		LC094041	**$A_1A_2A_6$(LC094001,LC094004,LC094005)**	**B_1/D_2(LC094011,LC094912)**	410982	
*D. erythrosora*4	Japan, Wakayama pref., Nachikatsuura-cho	20.66	3*x*		LC094041	$A_1A_2A_6$(LC094001,LC094004,LC094005)	**$B_1D_1D_2$(LC094009,LC094010,LC094011)**	411063	
*D. erythrosora*5	Japan, Tokyo-to, Hachioji-shi	24.80	3*x*	apo	LC094041	**$A_2A_4A_6$(LC094002,LC094003,LC094005)**	**$B_1D_1D_2$(LC094009,LC094010,LC094011)**	423511	
*D. hondoensis*1	Japan, Tokyo-to, Inagi-shi				LC094042	**$A_2A_3B_1$(LC094006,LC094007,LC094008)**	**$A_3B_2D_2$(LC094018,LC094037,LC094038)**	406833	
*D. hondoensis*2	Japan, Tokyo-to, Inagi-shi				LC094042	$A_2A_3B_1$(LC094006,LC094007,LC094008)	**$A_3B_1D_2$(LC094019,LC094036,LC094038)**	410248	
*D. hondoensis*3	Japan, Miyazaki pref., Kitakata-machi	22.91	3*x*		LC094042	**A_2/B_1(LC094006,LC094008)**	$A_3B_2D_2$(LC094018,LC094037,LC094038)	410346	
*D. hondoensis*4	Japan, Tokyo-to, Inagi-shi	20.49	3*x*		LC094042	$A_2A_3B_1$(LC094006,LC094007,LC094008)	$A_3B_1D_2$(LC094019,LC094036,LC094038)	410957	
*D. hondoensis*5	Japan, Tokyo-to, Inagi-shi	21.57	3*x*	apo	LC094042	$A_2A_3B_1$(LC094006,LC094007,LC094008)	**$A_3B_2D_2$(LC094018,LC094037,LC094038)**	410959	
*D. hondoensis*6	Japan, Wakayama pref., Shinguu-shi	20.82	3*x*		LC094042	$A_2A_3B_1$(LC094006,LC094007,LC094008)	**$A_3B_2D_2$(LC094018,LC094037,LC094038)**	410978	
*D. chinensis*1	Japan, Tokyo-to, Inagi-shi				AB908373*	**B_1/B_2 (AB908891*/AB908892*)**	*A_1*(LC094017)	405131	
*D. chinensis*2	Japan, Tokyo-to, Hachioji-shi				AB908373*	**B_1/B_2 (AB908891*/AB908892*)**	***A_1*(LC094017)**	405220	
*D. chinensis*3	Japan, Wakayama pref., Kozagawa-cho				AB908373*	**B_1/B_2 (AB908891*/AB908892*)**	*A_1*(LC094017)	405262	
*D. chinensis*4	Japan, Miyazaki pref., Kitakata-machi	13.01**	2*x***	sex**	AB908373	B_2B_2 (AB908892)	A_1A_1(LC094017)	410320	
*D. chinensis*5	Japan, Miyazaki pref., Hinokage-cho	13.85**	2*x***	sex**	AB908373	B_2B_2 (AB908892)	A_1A_1(LC094017)	410321	
*D. chinensis*6	Japan, Miyazaki pref., Nobeoka-shi	14.5**	2*x***	sex**	AB908373	B_2B_2 (AB908892)	A_1A_1(LC094017)	410342	
*D. chinensis*7	Japan, Miyazaki pref., Nobeoka-shi	13.56**	2*x***	sex**	AB908373	B1B2 (AB908891/AB908892)	**A_3A_3(LC094020)**	410350	
*D. chinensis*8	Japan, Miyazaki pref., Nobeoka-shi	13.22**	2*x***	sex**	AB908373	B_2B_2 (AB908892)	A_3A_3(LC094020)	410354	
*D. chinensis*9	Japan, Miyazaki pref., Nobeoka-shi	13.22**	2*x***	sex**	AB908373*	B_2B_2(AB908892*)	A_3A_3(LC094020)	412500	
*D. chinensis*10	Japan, Miyazaki pref., Hyuga-shi	13.56**	2*x***	sex**	AB908373	B_2B_2 (AB908892)	A_3A_3(LC094020)	410356	
D. kinkiensis	Japan, Shizuoka pref., Kawanehonn-cho				LC094044	**LC093908,LC093910**	**LC094015,LC094016**	406837	
D. kinkiensis	Japan, Nara pref., Kashihara-shi			sex	LC094044	**LC093909,LC093910**	**LC094015,LC094016**	409127	
D. kinkiensis	Japan, Saga pref., Saga-shi	29.86	4*x*	sex	LC094044	**LC093908,LC093910**	**LC094015,LC094016**	410378	
D. kinkiensis	Japan, Saga pref., Saga-shi			sex	LC094044	**LC093908,LC093910**	LC094015,LC094016	410379	
D. kinkiensis	Japan, Wakayama pref., Nachikatsuura-cho	27.60	4*x*	sex	LC094044	LC093908,LC093910	LC094015,LC094016	411046	
D. kinkiensis	Japan, Wakayama pref., Nachikatsuura-cho	26.77	4*x*	sex	LC094044	LC093908,LC093910	LC094015,LC094016	411052	
D. sordidipes	Japan, Kagoshima pref., Yakushima Is.				AB908372*	**AB908894***	**LC094025**	407057	
D. sordidipes	Japan, Kagoshima pref., Yakushima Is.				AB908372	**LC093900,LC093901**	**LC094026**	410918	
D. sordidipes	Japan, Kagoshima pref., Yakushima Is.				AB908372	**LC093904,LC093905**	**LC094023,LC094026**	410919	
D. sordidipes	Japan, Kagoshima pref., Yakushima Is.				AB908372	**AB908894**	**LC094023**	410920	
D. sordidipes	Taiwan, Taipei City, Urai				AB908372	AB908894	LC094025	423478	
D. sordidipes	Taiwan, Taipei City, Urai				AB908372	**LC094023,LC094024**	**LC094024,LC094026**	423482	
D. protobissetiana	Japan, Kagoshima pref., Yakushima Is.	14.63***	2*x****	sex***	AB914474*	**AB914476***	**LC094028,LC094029**	410906	
D. protobissetiana	Japan, Kagoshima pref., Yakushima Is.	15.61***	2*x****	sex***	AB914474*	**AB914475***	LC094028	410907	
D. protobissetiana	Japan, Kagoshima pref., Yakushima Is.	16.58***	2*x****	sex***	AB914474*	**AB914475***	LC094028	410913	
D. protobissetiana	Japan, Kagoshima pref., Yakushima Is.	15.05***	2*x****	sex***	AB914474*	**AB914475***	LC094028	410914	
D. protobissetiana	Japan, Kagoshima pref., Yakushima Is.			sex***	AB914474*	**AB914475***	LC094028	410915	
D. protobissetiana	Japan, Kagoshima pref., Yakushima Is.	15.22***	2*x****	sex***	AB914474*	**AB914475***	LC094028	410916	
D. protobissetiana	Japan, Kagoshima pref., Yakushima Is.	16.10***	2*x****	sex***	AB914474*	AB914476*	**LC094028**	410917	
D. protobissetiana	Japan, Kagoshima pref., Yakushima Is.			sex	AB914474	AB914475	LC094028	425899	
D. protobissetiana	Japan, Kagoshima pref., Yakushima Is.			sex	AB914474	**LC093906**	LC094028	417179	
D. protobissetiana	Japan, Kagoshima pref., Yakushima Is.			sex***	AB914474*	**AB914475***	**LC094028**		763335
D. saxifraga	Japan, Tokyo-to, Okutama-machi			sex*	AB908375*	**AB908878***	**LC094030**	405345	
D. saxifraga	Japan, Tokyo-to, Okutama-machi			sex*	AB908375*	**AB908878***	LC094030	405346	
D. saxifraga	Japan, Tokyo-to, Okutama-machi			sex	AB908375	AB908878	**LC094031**	405347	
D. saxifraga	Japan, Tokyo-to, Okutama-machi			sex	AB908375	AB908878	LC094030	405348	
D. saxifraga	Japan, Tokyo-to, Okutama-machi			sex	AB908375	AB908878	LC094030	405349	
D. saxifraga	Japan, Tokyo-to, Okutama-machi			sex	AB908375	AB908878	LC094031	411382	
D. saxifraga	Japan, Tokyo-to, Okutama-machi			sex	AB908375	AB908878	LC094031	411383	
D. saxifraga	Japan, Tokyo-to, Okutama-machi			sex	AB908375	AB908878	LC094030	411385	
D. saxifraga	Japan, Tokyo-to, Okutama-machi			sex	AB908375	AB908878	LC094030	411386	
D. saxifraga	Japan, Tokyo-to, Okutama-machi			sex	AB908375	AB908878	LC094030	411387	
D. saxifraga	Japan, Aichi pref., Shitara-cho			sex	AB908375	AB908878	LC094030	411406	
D. saxifraga	Japan, Akita pref., Senboku-shi			sex	AB908375	AB908878	LC094030	423578	
D. saxifraga	Japan, Akita pref., Senboku-shi			sex	AB908375	AB908878	LC094030	423579	
D. saxifraga	Japan, Akita pref., Senboku-shi			sex	AB908375	AB908878	LC094031	423584	
D. saxifraga	Japan, Akita pref., Senboku-shi			sex	AB908375	AB908878	LC094030	423586	
D. saxifraga	Japan, Akita pref., Senboku-shi			sex	AB908375	AB908878	LC094030	423587	
D. saxifraga	Japan, Akita pref., Senboku-shi			sex	AB908375	AB908878	LC094030	423588	
D. saxifraga	Japan, Akita pref., Senboku-shi			sex	AB908375	AB908878	LC094030	423590	
D. varia	Taiwan, New Taipei City, Pingling			sex	AB908376*	**AB908876***	**LC094027**		1181085
D. sabaei	Japan, Tokyo-to, Inagi-shi				LC095874	**LC093907**	**LC094021,LC094022**	406981	
D. gymnophylla	Japan, Tokyo-to, Hachioji-shi				AB908370*	**AB908893***	**LC094032**	405219	